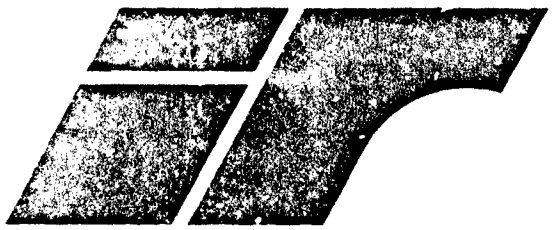

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PRECLINICAL STUDIES OF THE OXIME, HI-6, AN ELEMENT OF THE TREATMENT OF SOMAN POISONING-- Appendix 13, CR 26/85, The Pharmacokinetics of HI-6 in the Rat and Dog

Appendix to Final Report

by

C.J. Briggs and K.J. Simons

March 1982

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pharmacokinetics, bioavailability, absorption, excretion.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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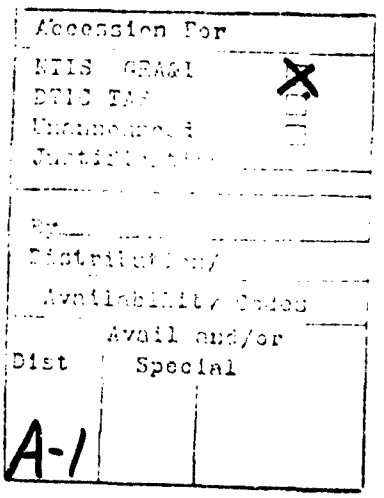

THE PHARMACOKINETICS OF HI-6

IN THE RAT AND DOG

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FINAL REPORT

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SUBMITTED BY: DR. C.J. BRIGGS AND DR. K.J. SIMONS,
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WINNIPEG, MANITOBA, R3T 2N2.

DATE: MARCH 31, 1982

TABLE OF CONTENTS

I. THIN-LAYER CHROMATOGRAPHY OF HI-6

INTRODUCTION:

Pharmacokinetic and drug distribution studies require accurate determination of dosage of a drug. Compounds under investigation should be pure, and if this is not technically feasible, the extent and nature of any impurity should be known. Many techniques are available for monitoring the quality of chemicals, but the most widely used are various forms of chromatography.

Pharmacokinetic studies on the cholinesterase reactivator, HI-6, involved the use of different batches of material[1] during the initial stages of the project. It was essential to check these samples for the presence of impurities or breakdown products. Thin-layer chromatography was selected as a sensitive, convenient procedure for monitoring the different batches of HI-6 for chromatographic purity. Use of two distinctly different types of plate provides the greatest opportunity for detection of impurities. Silica gel and Avicel (cellulose) were selected, since the former is primarily an adsorption medium whereas the latter is used in partition chromatography. Use of a variety of solvents on these media constituted a sensitive procedure for monitoring the purity of HI-6.

EQUIPMENT AND SUPPLIES:

Plates - Baker Precoated Silica Gel 250 µm, with fluorescent Indicator (254 nm).[2]

- Avicel Precoated Cellulose Plates 250 µm.[3]

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[1]Synthesized and purified by Dr. P. Lockwood, Defence Research Establishment Suffield.

[2]J.T. Baker Chemical Co., Phillipsburg, N.J. 08865, U.S.A.

[3]Analtech Laboratories, Supplied through Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA, 15219, U.S.A.

- Solvents - Ethanol-Commercial Alcohols Ltd.[4]
- All others Fisher ACS Grade.[5]

Chromatography Equipment - Desage TLC Supplies.[6]

Ultra violet light - Chromato-vue Cabinet with 365, 254 nm and visible light-Fisher Scientific.[7]

METHODS:

All thin-layer chromatography was performed using the supersaturation method of Stahl (1). In routine studies, plates were prewashed with developing solvent prior to use, and were reactivated by air-drying at room temperature overnight, prior to application of the compounds under investigation. Three separate solvent systems were used with Silica gel plates (20 x 20 cm, 250 µm thick, fluorescent at 254 nm).

Solvent System A: Ethanol-Ammonia (0.88)-Water (5:1:2);

Solvent System B: Ethyl Acetate-Ethanol (8:2);

Solvent System C: Methanol-Ammonia (0.88) (200:3) (ref 2);

Avicel (20 x 20 cm, 250 µm) Plates were developed using Solvent System D: Citric acid-Butanol-Water (0.48 g:87 ml:13 ml).

Preliminary studies involved concentration range-finding for HI-6 on the silica plates. The compound was dissolved in aqueous ethanol and applied to the plates. Weights of HI-6 used were in the range of 2-200 µg per spot.

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[4]Commercial Alcohols Ltd., Gatineau, P.Q.

[5]Fisher Chemicals, Fairlawn, Jew Jersey, 07410, U.S.A.

[6]Canlab Supplies, 80 Jutland Rd., Toronto, Ontario, M8Z 2M4.

[7]Fisher Scientific Co. Ltd., 8555 Devonshire Road, Montreal, P.Q., H4P 2L3.

A selection of different brands of silica gel plates was evaluated for the presence of interfering compounds used in the binder or preparation of the plates. Plates were developed in solvent systems described above, and examined under U.V. Visible impurities were noted. The value of pre-washing the plates with developing solvent, followed by re-activation by air drying at room temperature was demonstrated.

Samples of HI-6 were broken down by storing solutions at room temperature at a pH of 13 for 24 hours. Samples were taken and applied to thin-layer plates to demonstrate the ability of the systems to separate HI-6 from its breakdown products. The solutions were neutralized with hydrochloric acid prior to application to the plates.

The possibility of interference from plasma was checked in each solvent system using 50 µl samples applied prior to developing the plate.

RESULTS AND DISCUSSION:

Thin-layer chromatography plates frequently contain materials which interfere with chromatographic separation and detection of compounds under investigation. Commercially prepared plates may adsorb plasticizers and other components of the wrapping material, and other compounds may be eluted from the binder. In the present studies, it was found that a yellow contaminant was concentrated at the solvent front. This occurred with all systems on the silica gel (Baker) plates, and to a less significant extent with the Avicel. It was shown that the source of the contamination was independent of solvent and the problem was eliminated if the plates were prewashed with developing solvent and then reactivated.

Whatman K-6, Centra-Penta, B.D.H. and Merck Silica Gel C plates were all compared with the Baker plates with regard to the occurrence of this

contaminating material. None offered any advantage over the Baker plates in this regard, and several were significantly worse. The Baker plates were adopted for routine use. The Avicel plates were compared with MN-300 cellulose plates prepared locally, but reproducibility was less satisfactory with the latter, although contamination levels were lower. However, the Avicel plates were selected for monitoring purposes on the grounds of consistency, convenience and the ease with which they could be washed to eliminate contaminants.

The amount of HI-6 which can be applied to a plate is dependent upon the purpose for which the study is intended. If one is to use the plate to separate HI-6 from metabolites, breakdown products and impurities, then there must be clear separations and no streaking. However, if one seeks to detect other compounds present in the HI-6 then some streaking and incomplete separation may be acceptable since application of a greater quantity of HI-6 will simultaneously increase the amount of impurity present. This will facilitate detection of impurities.

The range finding study covered quantities from 1 µg - 200 µg per spot of reference HI-6, and the equivalent range of HI-6 which had been stored in basic solution (pH 13) for 24 hours. The optimum range for practical application was found to be 2 - 100 µg per spot. Excessive streaking of breakdown products occurred at the 200 µg level.

The solvent systems described here are modifications of published systems, and are applicable to the study of many cholinesterase reactivators. In our laboratories they have been used to study toxogonin and PAM Chloride. They are effective for separating breakdown products from the compounds under investigation. The cholinesterase reactivators have low Rf's in these systems,

whereas the impurities migrate to a significant extent. Typical separations have been described by Christenson (3). Her study of toxogonin stability employed TLC with silica plates and solvent systems similar to those used in the present study. The use of propanol in place of ethanol in the solvent did not significantly alter the separations obtained.

Pure HI-6 did not migrate in the systems described here, but the techniques were applicable to the study of contaminants and breakdown products. For many purposes, it would be desirable to move HI-6 from the origin. Acidic solvents are under investigation for use on cellulose plates. Various combinations of hydrochloric acid and methanol/water appear to be potentially useful for this purpose, and are currently under study.

With strongly basic solvent systems, there is evidence of some breakdown of HI-6 during chromatography. This is characterized by streaking, with no tendency to resolution into distinct spots, even when loading is insufficient to cause streaking of any impurities present in the starting material. Such breakdown was not found to be a significant problem with the solvents described in this paper. However, the use of an acidic solvent system would prevent breakdown of HI-6 which occurs on the plates after prolonged exposure to high pH systems.

The sample of HI-6 supplied for investigation purposes was shown to be chromatographically pure. The TLC systems were also shown to be appropriate for use in the presence of plasma, since this product did not interfere with detection of HI-6 or its breakdown products.

II. DETERMINATION OF HI-6 IN PLASMA AND URINE

INTRODUCTION:

In order to study the pharmacokinetics of HI-6, methods for measuring drug concentration in biological fluids must be developed. Serum and urine concentrations of some of the earlier aldoximes, e.g. PAM-Cl, TMB-4 and toxogonin were determined spectrophotometrically. The protein was precipitated with trichloroacetic acid or removed by dialysis and the absorption of the corresponding oximate ion was measured at 335 nm (4). These procedures have been modified for HS-6 (5) and HI-6 (6). Further studies with HS-6 (7) have shown that the H oximes are unstable in strongly alkaline solutions. Therefore buffers have been used to maintain a pH which ensures a suitable concentration of oximate ion, without severely affecting the stability of these compounds.

In order to develop methods for the determination of HI-6 in plasma and urine of rats and dogs, these current methods have been evaluated and modified.

METHODS:

Determination of HI-6 in Plasma.

(i) Small Animals, e.g. Rats.

Stock concentrations of HI-6[1] in water (20 mg/ml) were diluted with plasma to yield concentrations of 20, 40, 60, 80 and 100 µg/ml of drug. To assay for HI-6, 0.2 ml of stock plasma solution or test plasma sample were pipetted[2] into 10 x 75 mm test tubes[3] and diluted

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[1] Synthesized and purified by Dr. F. Lockwood, Defence Research Establishment Suffield, Ralston, Alberta, TOJ 2N0.

[2] Centaur Micropipette, Chromatographic Specialties Ltd., Brockville, Ont. K6V 5W1.

[3] Canlab, Toronto, Ontario, M8Z 2H4.

with 0.2 ml of 10% trichloroacetic acid[4]. The samples were mixed thoroughly[5] and centrifuged[6] for 20 min. From these tubes, 0.2 ml of supernatant was transferred to a clean tube and just prior to spectrophotometric analysis, diluted with 0.2 ml of 0.5 N ammonium hydroxide. Samples were mixed thoroughly, transferred to micro-cuvettes[7] and the absorbance at 355 nm[8] was determined immediately. The concentrations were determined from calibration curves constructed by plotting the absorbance of stock solutions versus HI-6 concentration. Plasma containing no drug, and treated similarly to the standards was used as the reference. Urine samples from rats were analyzed by the method for large animals.

(ii) Large Animals e.g. Dogs.

Standard solutions of HI-6[1] in plasma were prepared containing 25 to 150 µg/ml of drug. Accurately measured 1 ml samples of plasma from standard solutions or plasma or urine from dosed animals were pipetted[2] into 13 x 100 mm test tubes[3]. To these samples 1 ml of 10% trichloroacetic acid[4] was added. The samples were mixed on a vortex mixer[5] and centrifuged[6] for 20 min at 2000 rpm. The total supernatant was transferred to a clean dry 13 x 100 mm test tube[3]. Excess calcium carbonate[4] was added. The samples were mixed on a vortex mixer[5] and centrifuged[6] for 5 min. Exactly 1 ml[2] of supernatant was transferred to a clean test tube containing 4 ml of 0.05 M tris buffer pH 8.8[9]. The samples were mixed as before and centrifuged to

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[4]Fisher Scientific Co., Fair Lawn, N.J. 07410.

[5]Vortex-Genie, Scientific Industries Inc., Bohemia, N.Y.

[6]International Centrifuge, International Equipment Co., Boston, Mass.

[7]Arthur H. Thomas Company, Philadelphia, PA.

[8]Acta III, Beckman Instruments Inc., Fullerton, CA, 92634.

[9]Trizma 8.8, Sigma Chemical Co., St. Louis, MO 63178, U.S.A.

remove a precipitate. Samples were transferred to 10 mm cuvettes[7] and the absorbance at 355 nm[8] was measured. Plasma containing no drug, and treated similarly to the standards, was used as the reference for plasma samples. For urine samples it was impossible to obtain a control blank so water was used as the reference.

RESULTS AND DISCUSSION:

A typical spectrophotometric scan of a plasma sample of HI-6, precipitated with 10% TCA and made alkaline with 0.5 N ammonium hydroxide is shown in Figure 1-I. No absorbance over this wavelength range was detected in plasma, containing no HI-6, treated with TCA and ammonium hydroxide (Figure 1-II).

A mean calibration curve (average of 9 sets of standards) is shown in Figure 2. The maximum coefficient of variation is 6.7% over a 2 month period and the method is sufficiently sensitive to measure 10 µg/ml in 200 µl of serum or plasma. The calibration curve does not pass through the origin. However the correlation coefficients are nearly always 1.00, so linearity is acceptable. The reason that the line does not pass through the origin may be due to the fact that the micro-cuvettes have a smaller path width than the 10 mm cuvettes. Absorption by the cuvettes themselves may therefore affect the intercept of the calibration curve.

There is evidence that HS-6 is unstable in alkaline solutions (7) and that HI-6 is even more unstable. In a stability study that was carried out up to 30 min in 0.2N NaOH, the half-life of decomposition was 1.44 hr. However, when the plasma samples were made alkaline with 0.5 N ammonium hydroxide, the final pH values were ~ 8.95. When repeated absorbance measurements were taken from these duplicate samples, less than 3% loss of

absorbance occurred over 1 hr. To minimize even this loss, the 0.5 N ammonium hydroxide was added just prior to the spectrophotometric measurement.

A typical spectrophotometric scan of a serum sample of HI-6 analyzed using tris buffer is shown in Figure 3-I. No absorbance over this range is detected in plasma containing no HI-6 (Figure 3-II). Based on this scan, absorbance measurements are determined at 355 nm. A mean calibration curve (average of 14 sets of standards) is shown in Figure 4. The maximum coefficient of variation is 4.6% and the method is sufficiently sensitive to measure 25 µg/ml in 1 ml of serum or plasma. With this method the calibration curve passes through the origin.

This method was also satisfactory for the determination of HI-6 in urine to which the drug had been added *in vitro*. This method was therefore used for the determination of drug in the urine of dogs given a 20 mg/kg dose of HI-6. The control urines were not useful however as they were obtained overnight and appeared to contain interfering substances. Reproducible results were obtained from the HI-6 containing dog urine samples when water was used as a control. Therefore, there appeared to be no endogenous substances in these urine samples that would interfere with the assay.

The results of HI-6 recovery in rat urine were not as satisfactory. In this instance it was not possible to obtain control urine samples. When the amounts of HI-6 were determined using water as a reference, some recoveries exceeded 100%. For this reason, the method may not be sufficiently specific for the determination of HI-6 in rat urine.

SUMMARY AND CONCLUSIONS:

For the determination of HI-6 in plasma and urine, two methods were modified and developed. The method for blood samples from small animals where minimal volume could be obtained was specific, sensitive and reproducible. There was no interference from plasma components.

The method for samples obtained from larger laboratory animals was also specific sensitive and reproducible for plasma samples. There was no interference from plasma components. This method also worked well for the determination of HI-6 in urine from dogs. However, the method was not as satisfactory for the determination of HI-6 in urine from rats. This problem may be resolved by the development of HPLC assays.

III. THE PHARMACOKINETICS OF HI-6 IN DOGS AND RATS

INTRODUCTION:

The bispyridinium mono-oxime HI-6 has been found very effective in the treatment of laboratory animals poisoned with the nerve gas agent soman (8,9). Serum concentrations were not determined in these studies so the pharmacokinetics of HI-6 could not be calculated and effective serum concentrations could not be established.

The pharmacokinetics of some of the older bispyridinium bis-oximes TMB-4 and toxogonin have been studied in humans and dogs (10-13). Although toxogonin has been adminstered both intravenously (12) and intramuscularly (13), no attempts were made to determine the bioavailability of toxogonin following intramuscular injection.

To date, the pharmacokinetics of HI-6 have only been studied in rats following intravenous administration of the drug (6). We have studied the pharmacokinetics of HI-6 in dogs and rats following intravenous and intramuscular administration. We have also compared the effect of volume injected on the rate of absorption and bioavailability of HI-6 administered intramuscularly by using solutions at two different drug concentrations.

METHODS:

Doses:

The HI-6 was provided for these studies[1] and the purity was established in our laboratory. Solutions of HI-6 (25, 125 and 250 mg/ml) with 1.5% benzyl alcohol as preservative were prepared using water for injection[2]. The

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[1]Synthesized and purified by Dr. P. Lockwood, Defence Research Establishment Suffield, Ralston, Alberta, T0J 2N0.

[2]Baxter Laboratories of Canada Ltd., Malton, Ontario, L4V 1J3.

solutions were sterilized by filtration (0.22 mμ)[3] and independently tested for sterility[4] (Appendix 1).

Animals:

For this study, 7 pure-bred Beagle dogs[5] (9.0±0.4 kg) and 35 out-bred Sprague Dawley 200-250 g rats[6] were purchased.

Dose Administration for Dog Study:

Each dog received a 20 mg/kg dose of HI-6 by intravenous (250 mg/ml solution) into the femoral vein or by intramuscular administration (25 and 250 mg/ml solutions) into the thigh muscle according to the randomized schedule (Table I). Animals were allowed free access to food and water right up to the time of study. Studies in each animal were at least 1 week apart.

Blood and Urine Sampling for Dog Study:

Prior to dose administration, a heparin lock[7] was inserted into the alternate femoral vein and a control blood sample was drawn. A control overnight urine specimen was also collected.

Following the intravenous dose administration, 3 ml blood samples were drawn at 2, 7, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min. Following the intramuscular dose administration, samples were drawn at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min. The plasma was separated and frozen until the samples could be analyzed. Pooled overnight urine was collected, the volume measured and an aliquot frozen for analysis.

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[3]Millipore Corporation, Bedford, Mass., 07130.

[4]St. Boniface General Hospital, 409 Tache Avenue, Winnipeg, Manitoba, R2H 2A6.

[5]Hazelton Research Animals Inc., Box 5, Cumberland, Virginia, 23040.

[6]Central Animal Care Service, University of Manitoba (Gunton), Winnipeg, Manitoba, R3E 3J7.

[7]Butterfly-21 Infusion Set, Abbott Labs., N. Chicao, Ill., 60064.

Dose Administration for Rat Study:

Each rat received a 20 mg/kg dose of HI-6 by IV administration (125 mg/ml solution) into the dorsal vein of the penis, or by intramuscular administration (25 and 125 mg/ml solution) into the thigh muscle, according to the randomized dosing schedule (Table II). Animals were allowed free access to food and water right up to the time of each study.

Blood and Urine Sampling for Rat Study:

Due to the small size of the study animal, the volume of blood required for each sample and the number of blood samples required, 2 animals were used for each study. The animals were anesthetized with ether and the jugular vein was cannulated[8] and maintained with a heparin[9] lock. The 20 mg/kg dose of HI-6 was then administered. In the first rat of the pair under study (subscript 1) 0.5 ml of blood was collected at 3, 7, 10, 15, 30 and 90 min after dose administration. In the second rat (subscript 2) 0.5 ml of blood was collected at 30, 45, 60, 90, 120 and 150 min after dose administration. Animals were lightly anesthetized with ether during the entire study. The volume of blood withdrawn was replaced by an equal volume of 0.9% normal saline[2]. After the last blood sample the cannula was flushed and sealed. The animals were allowed to recover then placed in metabolism cages where urine was collected for at least 8 hr.

The plasma was separated in capillary blood serum separator tubes[10] and frozen until the samples could be analyzed. The urine was well-mixed, the volume recorded and an aliquot frozen for analysis.

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[8]Intramedic Polyethylene Tubing, 0.76 mm. i.d., 1.22 mm., o.d. (Clay Adams 7400), Fisher Scientific Co., Fair Lawn, J.J., 07410.

[9]Hepaleane (Heparin Sodium Injection U.S.P.), Harris Laboratories, Montreal, Quebec, H3E 1H4.

[10]Microtainer, Becton-Dickinson and Co., Rutherford, N.J., 07070.

HI-6 Assay:

The plasma and urine HI-6 concentrations were determined by spectrophotometry using the methods previously described.

Data Analysis: (14)

Plasma HI-6 concentration versus time plots from the intravenous dose for each animal were fitted to equation 1:

1) $Cp = Ae^{-\alpha t} + Be^{-\beta}$

where Cp is the plasma concentration at any time t and A, B, were the intercepts of the line. The constants α and β were the slopes of the line and represent the composite rate constants of distribution and elimination. Plasma HI-6 concentration versus time plots from the intramuscular doses were fitted to equation 2:

2) $Cp = A(e^{-Ket} - e^{-kat})$

where ka and Ke were the slopes of the lines and represent the rate constants of absorption and elimination respectively. The best possible fit to each set of experimental data was calculated using the BMDP[7] program on an AMDAHL V/7 computer.

Absorption half-lives ($t_{\frac{1}{2}_{abs}}$) of HI-6 following the intramuscular doses were calculated using equation 3:

3) $t_{\frac{1}{2}_{abs}} = \frac{\ln 2}{ka}$

and elimination half-lives ($t_{\frac{1}{2}}$) of HI-6 following the intravenous doses were calculated using equation 4:

4) $t_{\frac{1}{2}} = \frac{\ln 2}{\beta}$

and following the intramuscular doses using equation 5:

5) $t_{\frac{1}{2}} = \frac{\ln 2}{Ke}$

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[7]BMDP-77, University of Calif. Press, Berkeley, 1977.

Total body clearance (Cl) was calculated using equation 6:

$$6) \quad Cl = \frac{Dose}{\int_0^\infty Cpdt}$$

where $\int_0^\infty Cpdt$ represented the area under the plasma concentration versus time curve. This area was calculated using the trapezoidal rule to Cp_n. Extrapolation to time infinity was achieved by adding the value Cp_n/β or Cp_n/Ke.

The apparent volume of distribution (Vd) was calculated using equation 7:

$$7) \quad Vd = \frac{Cl}{Ke} \text{ or } \frac{Cl}{\beta}$$

Renal clearance (Cl_R) was calculated using equation 8:

$$8) \quad Cl_R = f.Cl$$

where f is the fraction of the dose of HI-6 excreted as unchanged drug in the urine.

Statistical analysis and comparison of the various pharmacokinetic parameters obtained following the intravenous and two intramuscular doses of HI-6 was conducted by using the paired and unpaired Student t test for the study in dogs and rats respectively (15). Statistical calculations were performed on an HP-67 programmable calculator[8] and differences were considered to be significant if $p \leq 0.05$.

RESULTS AND DISCUSSION:

Dog Studies:

The plasma concentrations of HI-6 in the 7 dogs following the 20 mg/kg intravenous dose are shown in Table III and following the concentrated and dilute intramuscular doses are shown in Tables IV and V respectively. The log mean ± sd HI-6 plasma concentrations versus time plots are shown in

[8]Hewlett Packard, 1000 N.E. Circle Blvd., Corvallis, OR 97330.

Figure 5 for the intravenous dose and in Figures 6 and 7 for the concentrated and dilute intramuscular doses respectively. The various pharmacokinetic parameters calculated following the intravenous dose are shown in Table VI and following the concentrated and dilute intramuscular doses, in Tables VII and VIII respectively.

The HI-6 plasma concentration versus time curve obtained after the intravenous administration of 20 mg/kg of drug to dogs was biexponential (Figure 5) so the two-compartment pharmacokinetic model was used for data analysis. Mean initial concentrations were 93.1±10.8 µg/ml falling to 8.7±2.4 µg/ml after 2 hr (Table III). The mean distribution half-life of 6.3±6.7 min was probably skewed by the 21 min value in dog 1. However in most dogs, distribution was complete in about 15 to 20 min. The mean elimination half-life of HI-6 in dogs was 48.2±17.7 min (Table VI). This is longer than the values of 28.3 min for TMB-4 and 19.9 min for toxogonin reported by other investigators (11). However, it still means that HI-6 blood concentrations will be negligible 4 hr after a dose.

The mean total body clearance of HI-6 in dogs following a 20 mg/kg intravenous dose was 5.16±0.81 ml/min/kg and the mean apparent volume of distribution was 0.37±0.20 l/kg (Table VI). Of the dose administered, 61.2±14.6% was excreted as unchanged drug over a 16 hr period following dose administration. For HI-6, with a plasma half-life of 48.2±17.7 min, this can be assumed to be the total fraction that would be excreted to infinite time. Therefore, renal clearance accounts for about 60% of a dose. However, it was not possible to detect metabolites with the analytical method used in this study.

The HI-6 plasma concentration versus time curves following the two

intramuscular doses are represented in Figures 6 and 7. Since the absorption phase completely masked the distribution phase, the results were calculated using the one compartment pharmacokinetic model with first-order absorption and elimination.

The pharmacokinetic parameters obtained following the concentrated and dilute intramuscular doses are shown in Tables VII and VIII respectively. There was no significant difference ($p = 0.05$) in the values obtained for half-life, clearance, apparent volume of distribution, per cent excreted unchanged, and renal clearance following the two intramuscular doses from those values obtained following the intravenous dose. The values obtained for the areas under the HI-6 plasma concentrations versus time curves following each dose are shown in Tables VI, VII and VIII repectively. There was no significant difference ($p = 0.05$) in the values obtained following either intramuscular dose from that obtained following the intravenous dose. From these comparisons it can be determined that virtually 100% of an intramuscular dose is absorbed. This means that the amount of drug absorbed by the body following an intramuscular dose is equivalent to an intravenous dose. It can be assumed that there are no problems with HI-6 bioavailability following an intramuscular dose.

Although there is no effect of the route of administration on the extent of absorption when HI-6 is injected intramuscularly, the effect of diluting the dose on the rate of absorption was evaluated. The mean absorption half-life of 8.0±3.0 min following the concentrated dose was not significantly different ($p = 0.05$) from the value of 5.9±2.2 min obtained following the diluted dose. The other parameters such as half-life, area under the curve, clearance, volume of distribution, fraction excreted unchanged and renal

clearance were not significantly different ($p = 0.05$) following either the concentrated or dilute dose.

Rat Studies:

The plasma concentrations of HI-6 in 10 rats given a 20 mg/kg intravenous dose are shown in Table IX. The plasma concentrations of HI-6 in 11 rats following the concentrated intramuscular dose and in 10 rats following the dilute intramuscular dose are shown in Tables X and XI respectively. The log mean ± sd HI-6 plasma concentrations versus time plots are shown in Figure 8 for the intravenous dose and in Figures 9 and 10 for the concentrated and dilute intramuscular doses respectively. The various pharmacokinetic parameters calculated following the intravenous dose are shown in Table XII and following the concentrated and dilute intramuscular doses, in Figures XIII and XIV respectively.

The HI-6 plasma concentration versus time curve obtained after the intravenous administration of 20 mg/kg of drug to rats was biexponential (Figure 8) so the two-compartment pharmacokinetic model was used for data analysis. If the HI-6 plasma concentrations were similar in the 2 rats comprising a study pair, all the data were analyzed as if from 1 animal. If there was a noticeable difference either in the concentrations or the trend of the data, the results were calculated for each individual rat.

Mean initial HI-6 plasma concentrations were 140.5±42.4 µg/ml falling to 14.9±7.5 µg/ml after 2.5 hr (Table IX). The mean distribution half-life of 4.1±1.3 min was similar to that of 7 min reported previously by other investigators (16).

The mean elimination half-life in rats was 65.6±21.0 min (Table XII).

This is longer than the values of 41 min (16) and approximately 30 min (6) following a 50 mg/kg intravenous dose to rats. However, in one study (16) the rats were anesthetized with pentobarbital, whereas in the present study they were lightly anesthetized with ether. The half-life following a 200 mg/kg dose was reported to be 101.9 min (6). From the currently available information, it is not possible to determine whether dose-dependent kinetics are found with HI-6. In the present study, with a mean half-life of about one hour, it can be calculated that blood concentrations would be negligible after 5 hours.

The mean total body clearance of HI-6 in rats following a 20 mg/kg intravenous dose was 3.95±0.93 ml/min/kg and the mean apparent volume of distribution was 0.38±0.17 l/kg (Table XII). Of the dose administered, 77.8±16.2% was excreted as unchanged drug. This is similar to results of 50% found in rats following a 50 mg/kg dose where HI-6 concentrations were measured by HPLC (16). However, since in at least 1 rat a value of 104.8% was obtained, the specificity of the analytical method for the determination of HI-6 in rat urine may be questionable. However, for completeness, renal clearance values were calculated using this method.

The HI-6 plasma concentration versus time curves following the two intramuscular doses are represented in Figures 9 and 10. As was found in the dog study, the absorption phase completely masked the distribution phase. The results were calculated using the one-compartment pharmacokinetic model with first-order absorption and elimination.

The pharmacokinetic parameters obtained following the concentrated and dilute intramuscular doses are shown in Tables XIII and XIV respectively. There was no significant difference ($p = 0.05$) in the values obtained for

half-life, clearance and apparent volume of distribution following the two intramuscular doses from those values obtained following the intravenous dose. The fractions of the dose excreted as unchanged drug and renal clearance values were not compared due to questionable values that may be due to the lack of assay specificity in these samples.

The values obtained for the areas under the HI-6 plasma concentration versus time curves following each dose are shown in Tables XII, XIII and XIV respectively. There was no significant difference ($p = 0.05$) in the values obtained following either intramuscular dose with that obtained following the intravenous dose. These results confirm those found in the dog study, that the intramuscular route of administration does not reduce the bioavailability of HI-6.

The mean absorption half-life of 4.6±3.1 min obtained from the concentrated intramuscular dose was not significantly different ($p = 0.05$) from the value of 9.2±5.3 min obtained following the diluted dose. Although the mean value obtained following the dilute dose is numerically twice that obtained from the concentrated dose, the large standard deviation does not permit a conclusive comparison in this small number of animals. The other parameters such as half-life, clearance and apparent volume of distribution were also not significantly different ($p = 0.05$) following either intramuscular dose.

SUMMARY AND CONCLUSIONS:

The pharmacokinetics of HI-6 have been studied in the dog and rat using one intravenous dose, a concentrated and a dilute intramuscular dose of 20 mg/kg. The concentrations of HI-6 were measured by a spectrophotometric method which was sufficiently sensitive and specific for all but rat urine samples. The half-life of HI-6 in dogs and rats is short, 48 and 66 min respectively. The intramuscular route of administration does not affect the extent of absorption when compared to the intravenous dose. Diluting the intramuscular dose has no effect on the rate or extent of absorption.

Thus, a dose of HI-6 is rapidly and completely absorbed following intramuscular administration.

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LEGENDS FOR FIGURES:

Figure 1. Spectrophotometric scan of HI-6 in 0.5 N ammonium hydroxide. I Plasma sample containing 100 µg/ml HI-6. II Control plasma sample.

Figure 2. Calibration curve constructed by plotting mean ± s.d. absorbance at 355nm versus HI-6 concentration using 0.2 ml of plasma and 0.5 N ammonium hydroxide.

Figure 3. Spectrophotometric scan of HI-6 in Tris Buffer pH 8.5. I Plasma sample containing 100 µg/ml HI-6. II Control plasma sample.

Figure 4. Calibration curve constructed by plotting mean ± s.d. absorbance at 355 nm versus HI-6 concentrations using 1 ml of plasma and Tris Buffer.

Figure 5. Mean ± s.d. log plasma HI-6 concentrations versus time plot following an intravenous dose of 20 mg/kg to 7 dogs. (—best computer-fitted line to mean data).

Figure 6. Mean ± s.d. log plasma HI-6 concentrations versus time plot following a concentrated (250 mg/ml) intramuscular dose of 20 mg/kg to 7 dogs (—best computer-fitted line to mean data).

Figure 7. Mean ± s.d. log plasma HI-6 concentrations versus time plot following a dilute (25 mg/ml) intramuscular dose of 20 mg/kg to 7 dogs (—best computer-fitted line to mean data).

Figure 8. Mean ± s.d. log plasma HI-6 concentrations versus time plot following an intravenous dose of 20 mg/kg to 10 rats (—best computer-fitted line to mean data).

Figure 9. Mean ± s.d. log plasma HI-6 concentrations versus time plot following a concentrated (125 mg/ml) intramuscular dose of 20 mg/kg to 11 rats (—best computer-fitted line to mean data).

Figure 10. Mean ± s.d. log plasma HI-6 concentrations versus time plot following a dilute (25 mg/ml) intramuscular dose of 20 mg/kg to 10 rats (—best computer-fitted line to mean data).

Figure 1

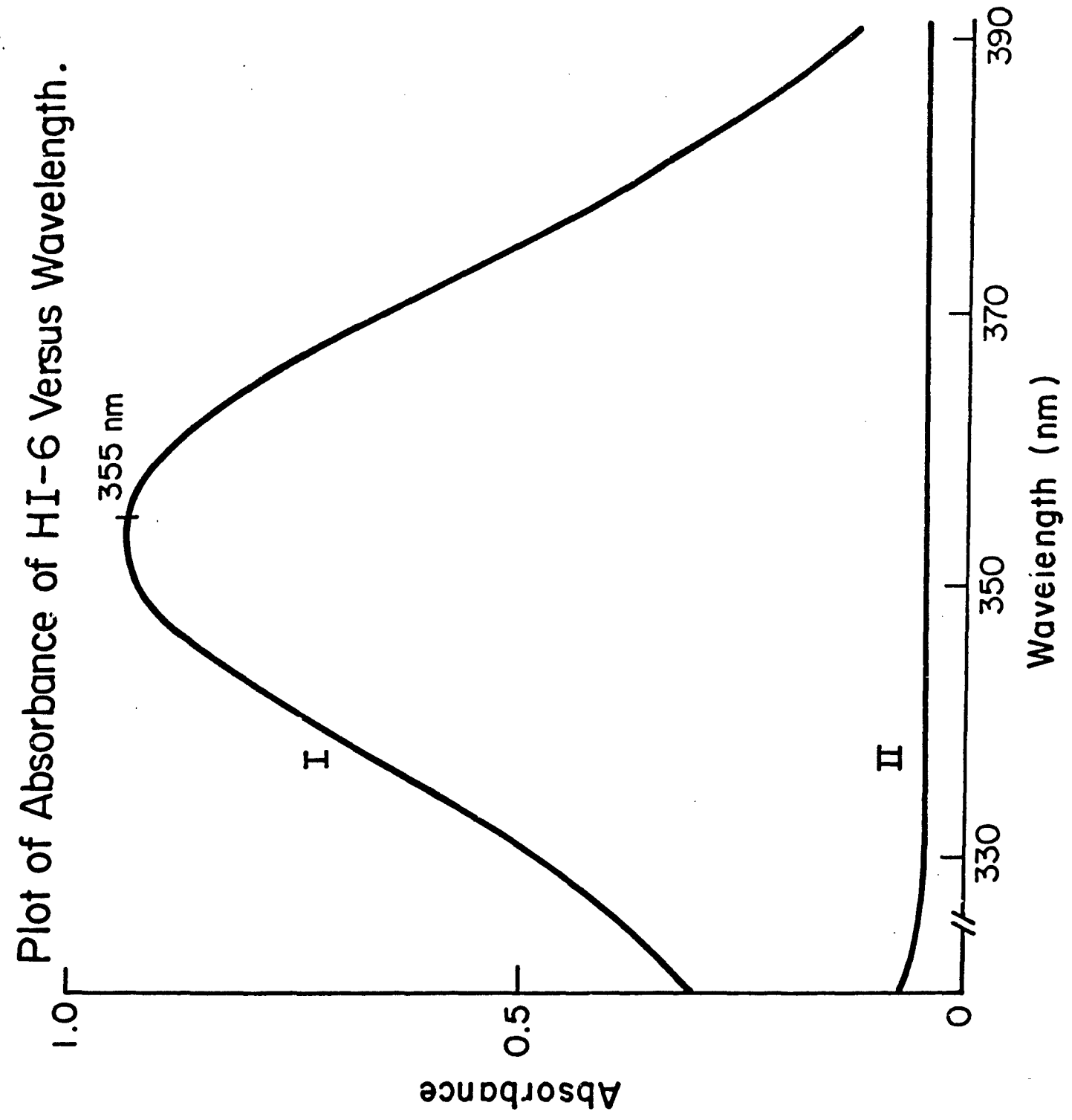
Plot of Absorbance of HI-6 Versus Wavelength.
355 nm
I
II
1.0
0.5
0
330
350
370
390
Wavelength (nm)
Absorbance

Figure 2

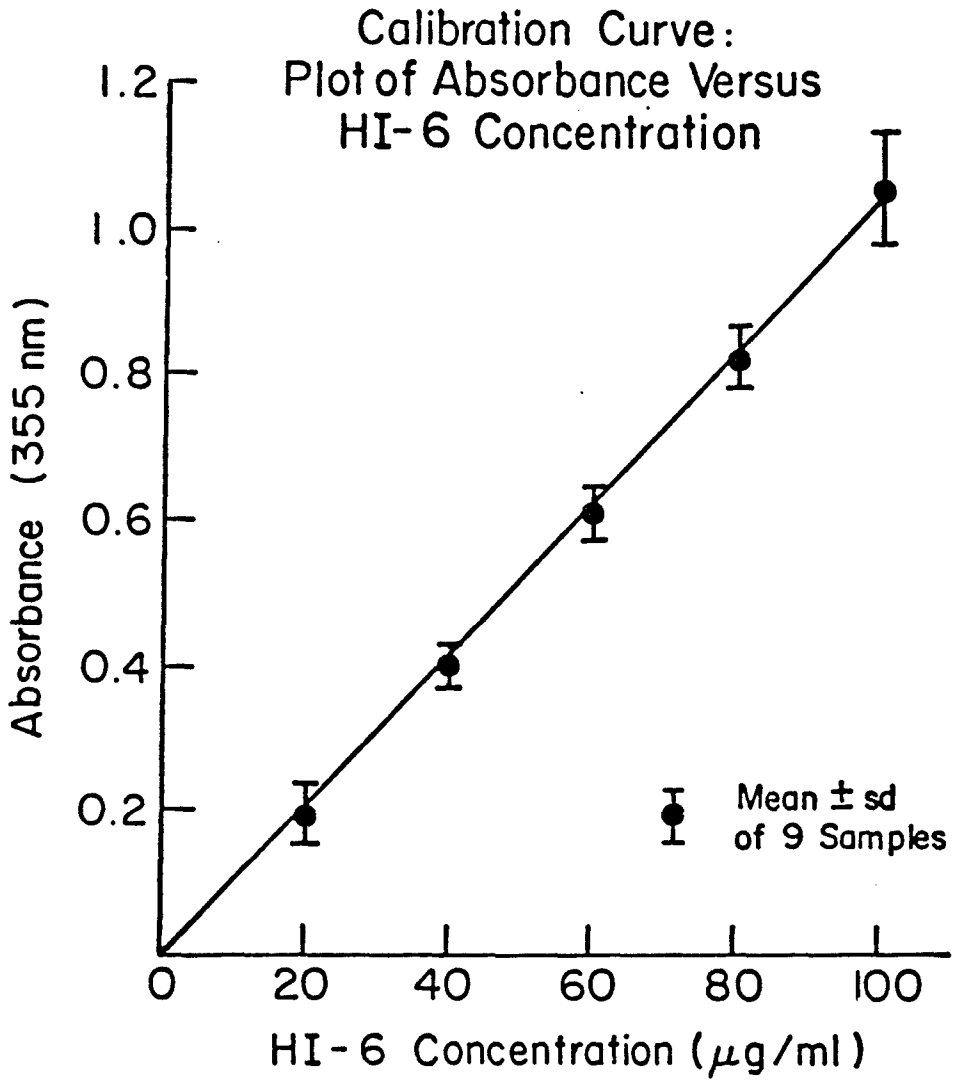
Calibration Curve:
Plot of Absorbance Versus
HI-6 Concentration
Absorbance (355 nm)
1.2
1.0
0.8
0.6
0.4
0.2
Mean ± sd
of 9 Samples
0
20
40
60
80
100
HI-6 Concentration (μg/ml)

Figure 3

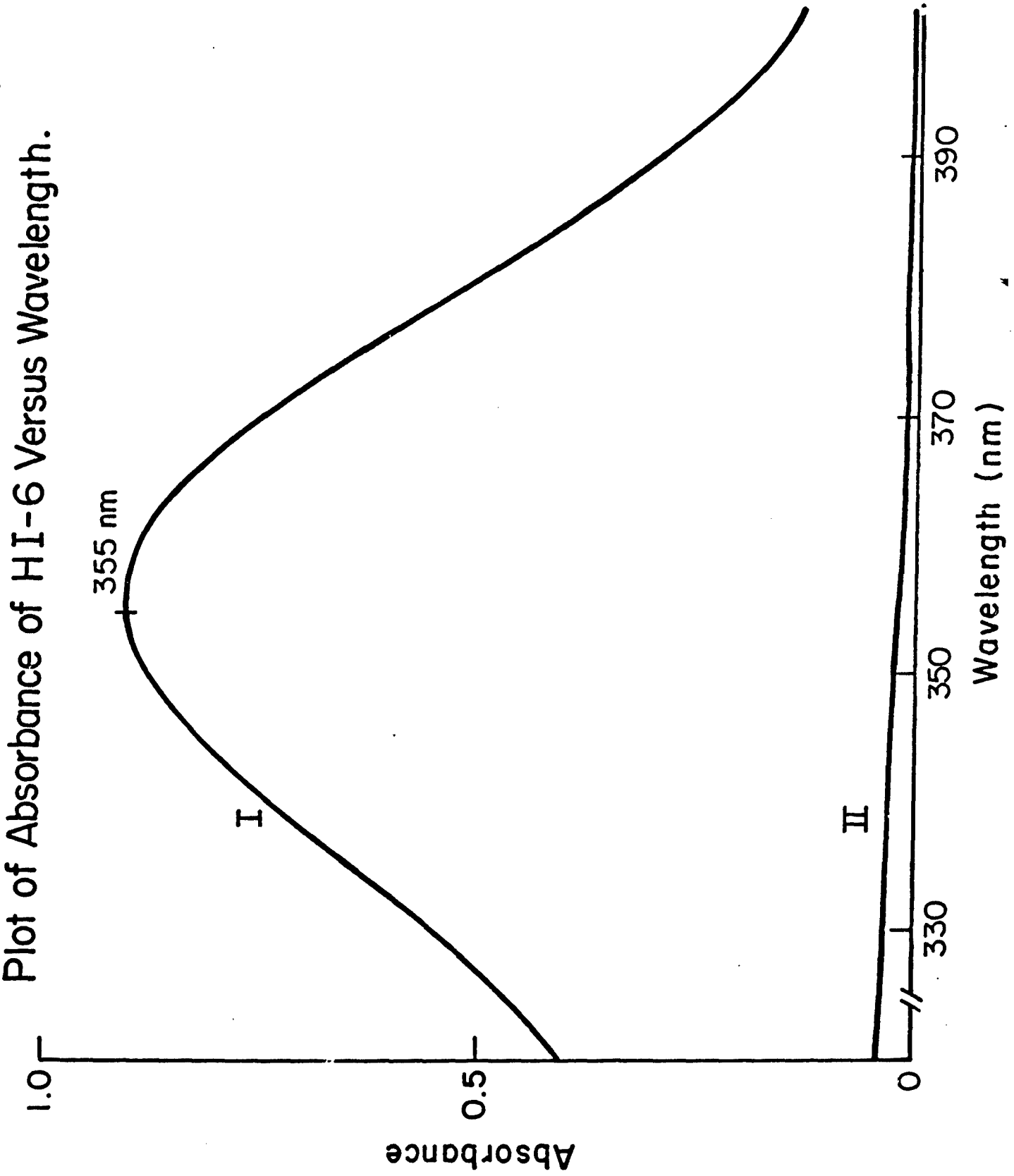
Plot of Absorbance of HI-6 Versus Wavelength.
355 nm
I
II
1.0
0.5
0
Absorbance
330
350
370
390
Wavelength (nm)

Figure 4

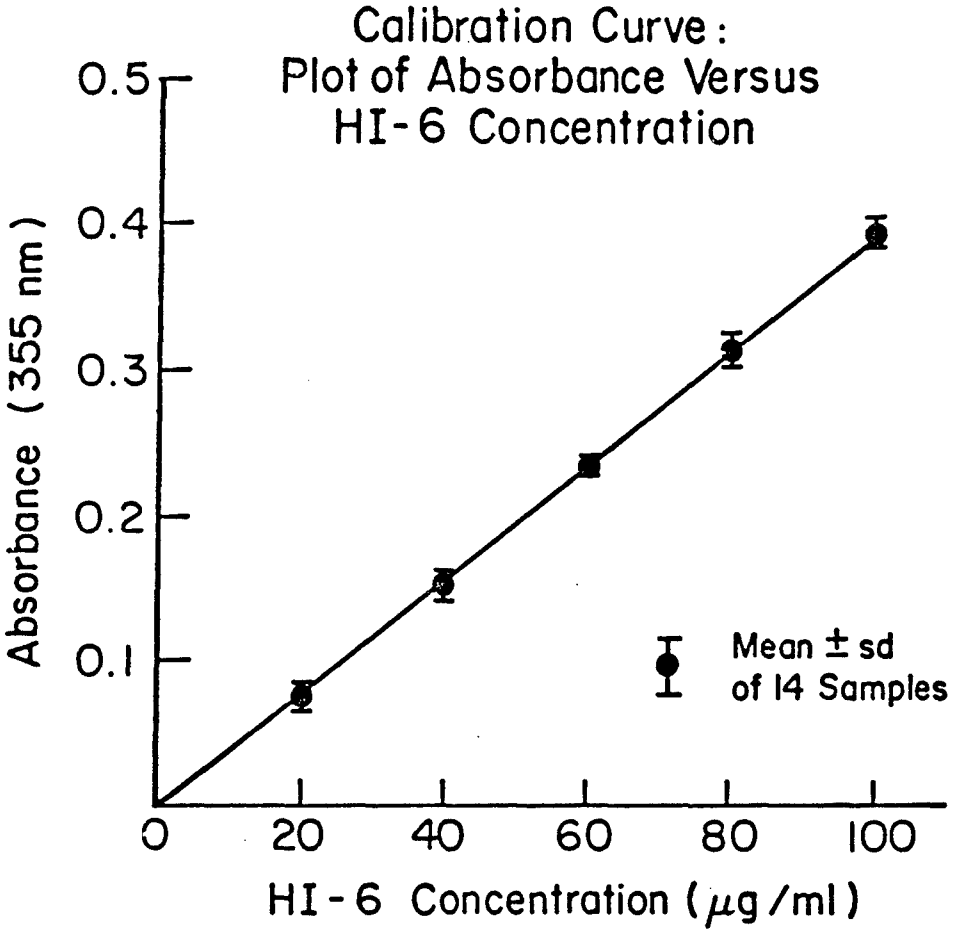
Calibration Curve:
Plot of Absorbance Versus
HI-6 Concentration
Absorbance (355 nm)
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
HI-6 Concentration (μg/ml)
Mean ± sd
of 14 Samples

Figure 5

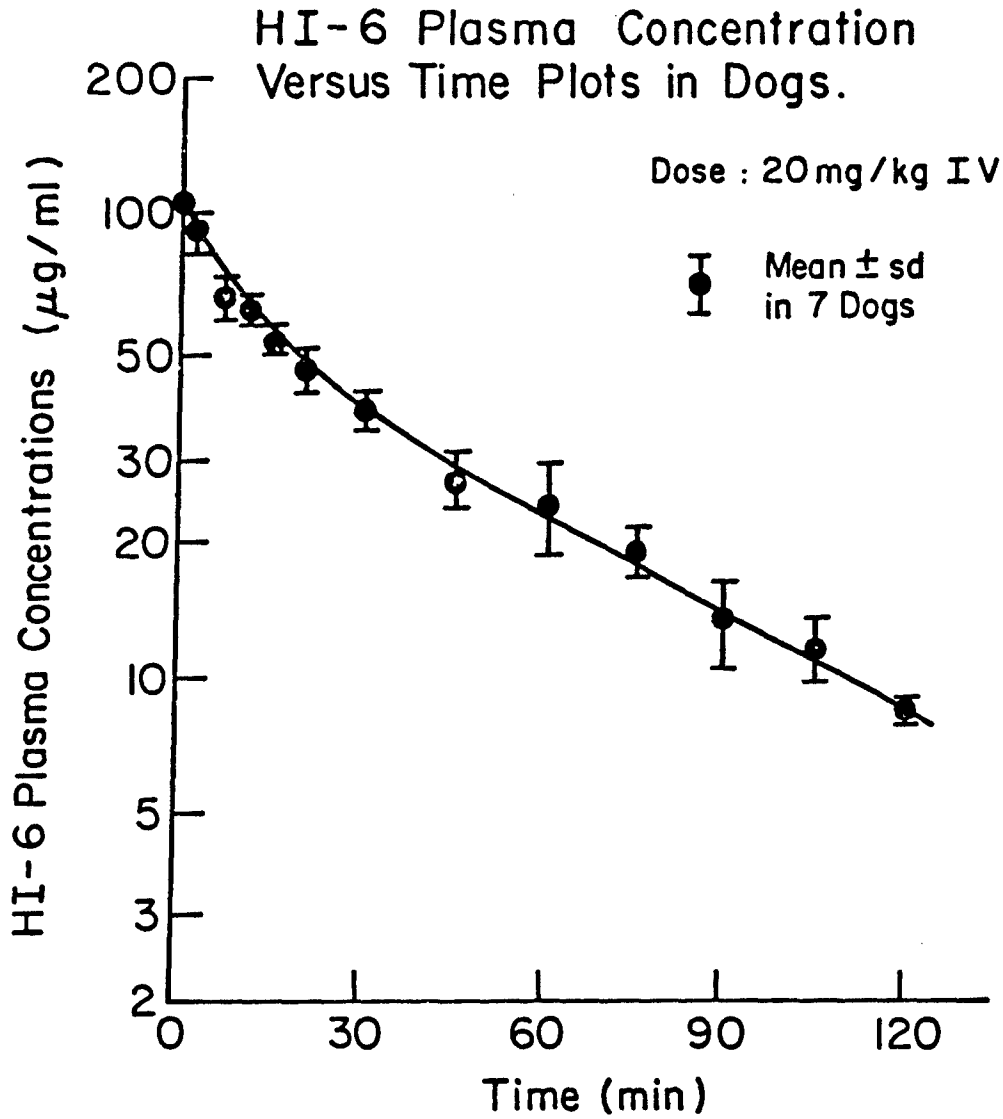
HI-6 Plasma Concentration
Versus Time Plots in Dogs.
Dose : 20 mg/kg IV
Mean ± sd
in 7 Dogs
HI-6 Plasma Concentrations (μg/ml)
200
100
50
30
20
10
5
3
2
0
30
60
90
120
Time (min)

Figure 6

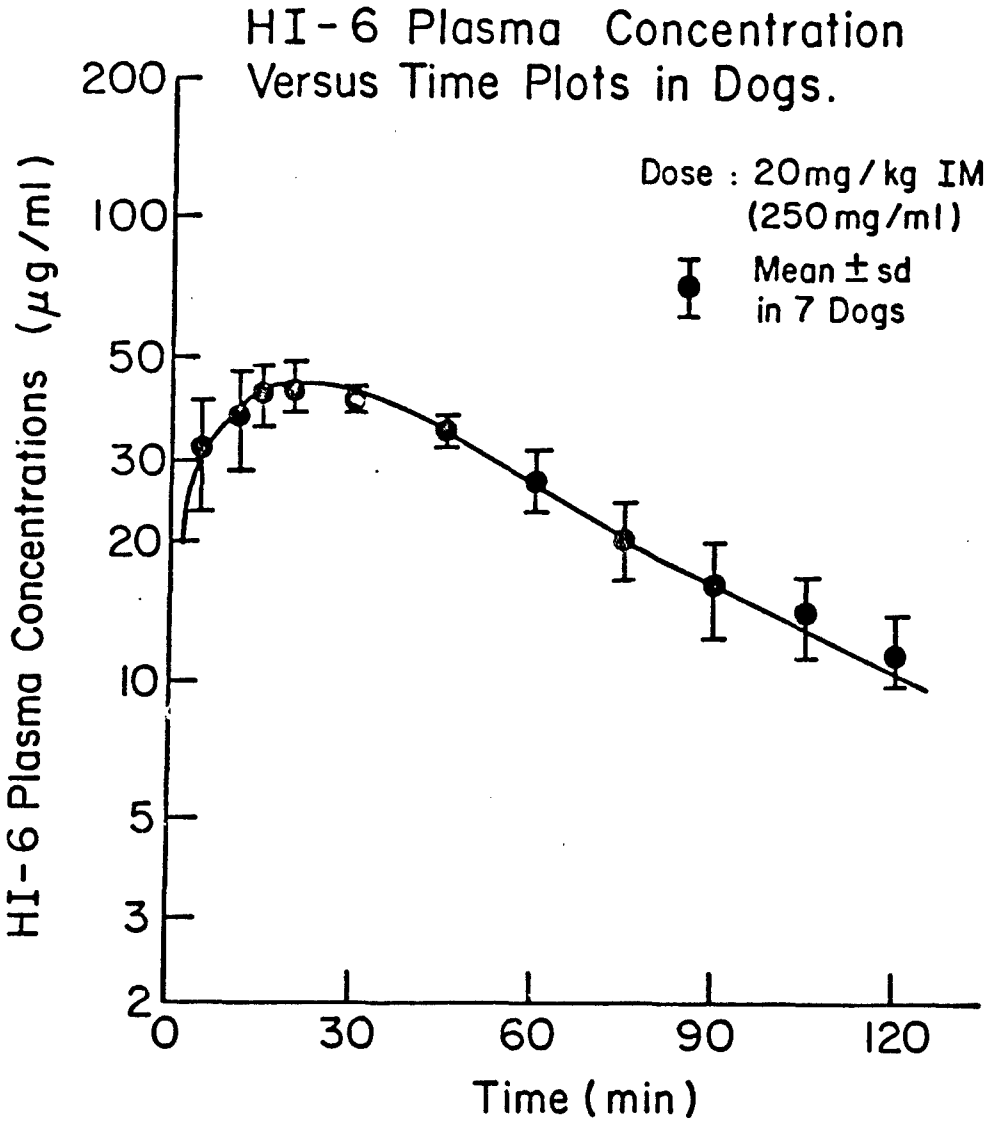
HI-6 Plasma Concentration
Versus Time Plots in Dogs.
Dose : 20mg / kg IM
(250 mg/ml)
Mean ± sd
in 7 Dogs
HI-6 Plasma Concentrations (µg/ml)
200
100
50
30
20
10
5
3
2
0
30
60
90
120
Time (min)

Figure 7

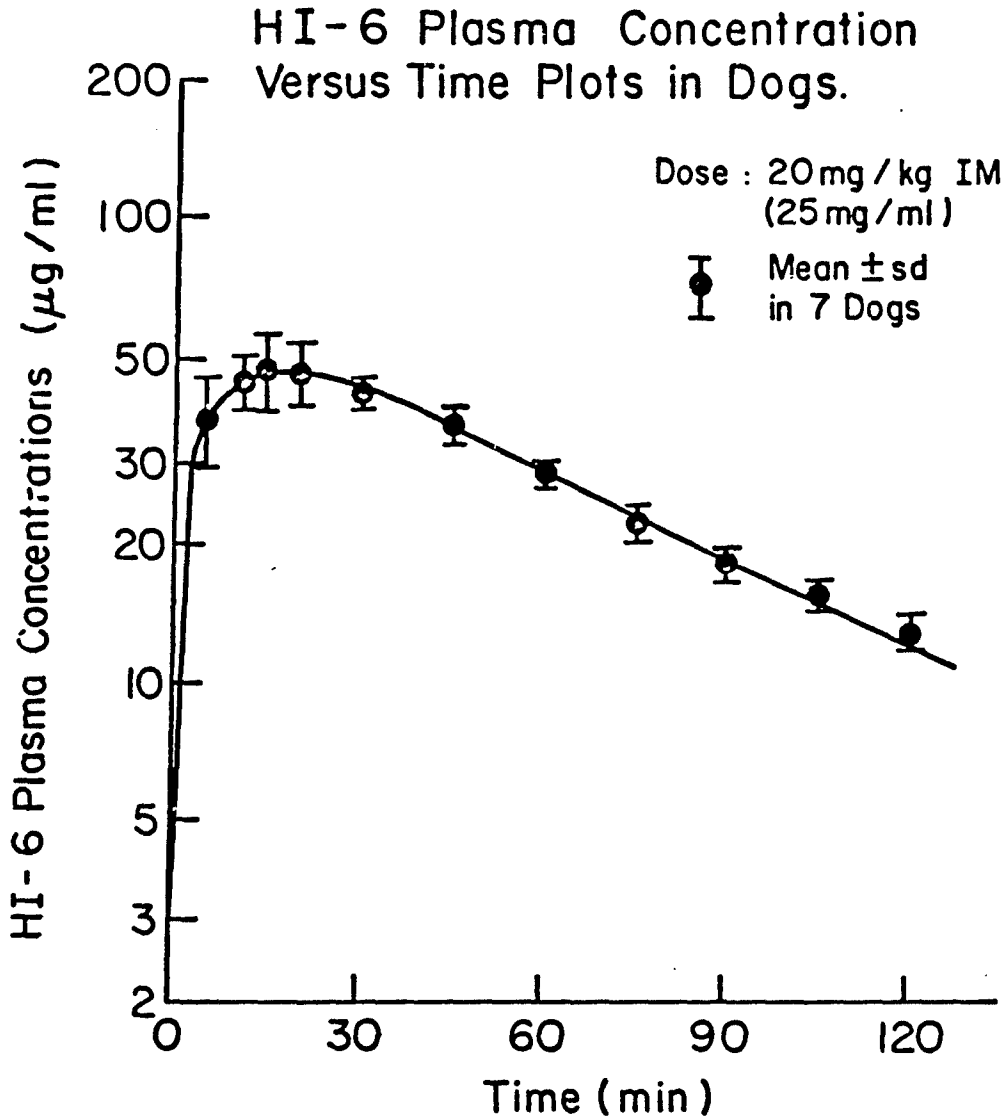
HI-6 Plasma Concentration
Versus Time Plots in Dogs.
Dose : 20mg/kg IM
(25mg/ml)
Mean ± sd
in 7 Dogs
HI-6 Plasma Concentrations (μg/ml)
200
100
50
30
20
10
5
3
2
0
30
60
90
120
Time (min)

Figure 8

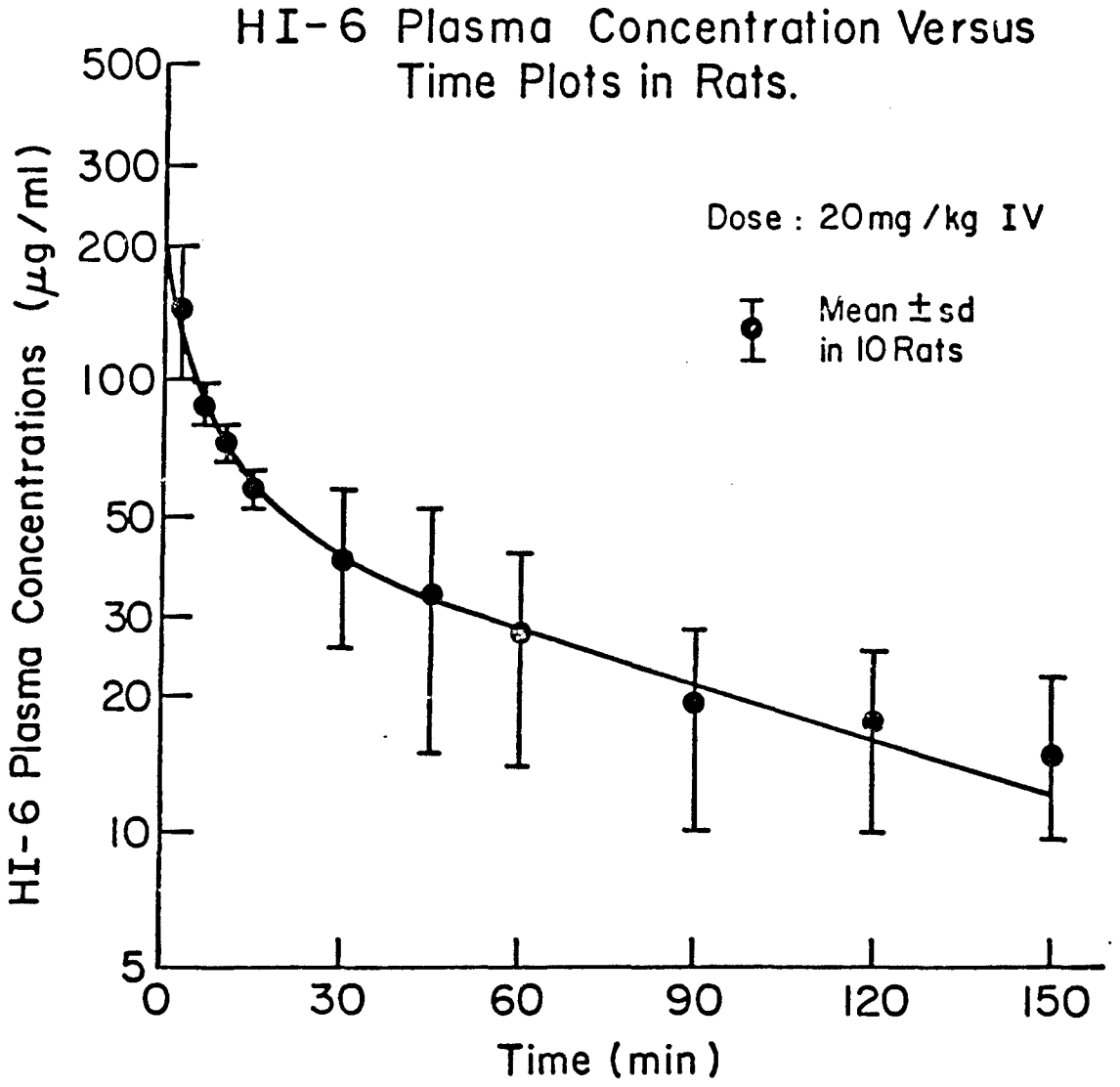
HI-6 Plasma Concentration Versus Time Plots in Rats.
Dose : 20mg /kg IV
Mean ± sd in 10 Rats
HI-6 Plasma Concentrations (μg/ml)
500
300
200
100
50
30
20
10
5
0
30
60
90
120
150
Time (min)

Figure 9

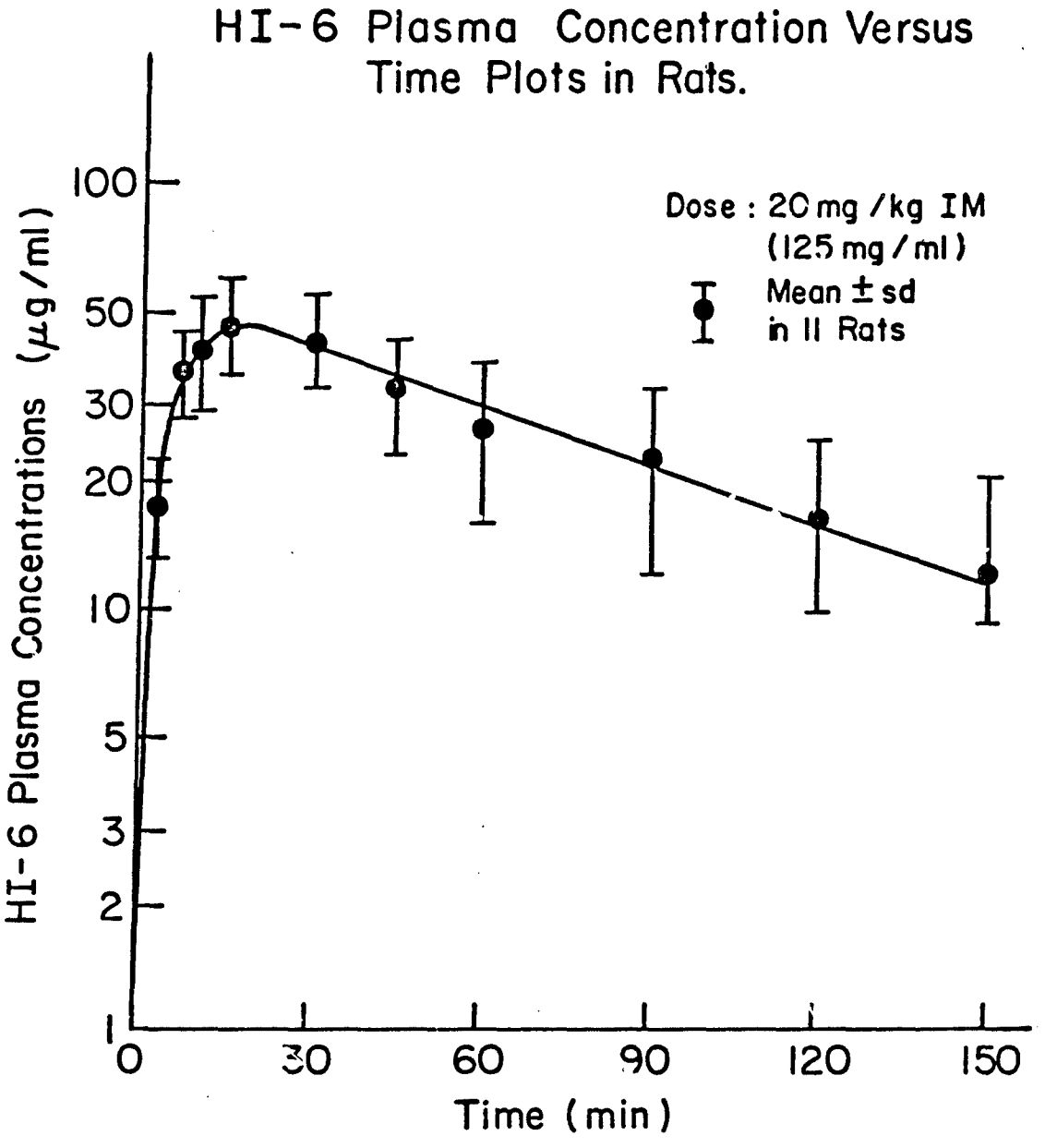
HI-6 Plasma Concentration Versus Time Plots in Rats.
HI-6 Plasma Concentrations (µg/ml)
100
50
30
20
10
5
3
2
1
0
30
60
90
120
150
Time (min)
Dose : 20 mg /kg IM
(125 mg / ml)
Mean ± sd
in 11 Rats

Figure 10

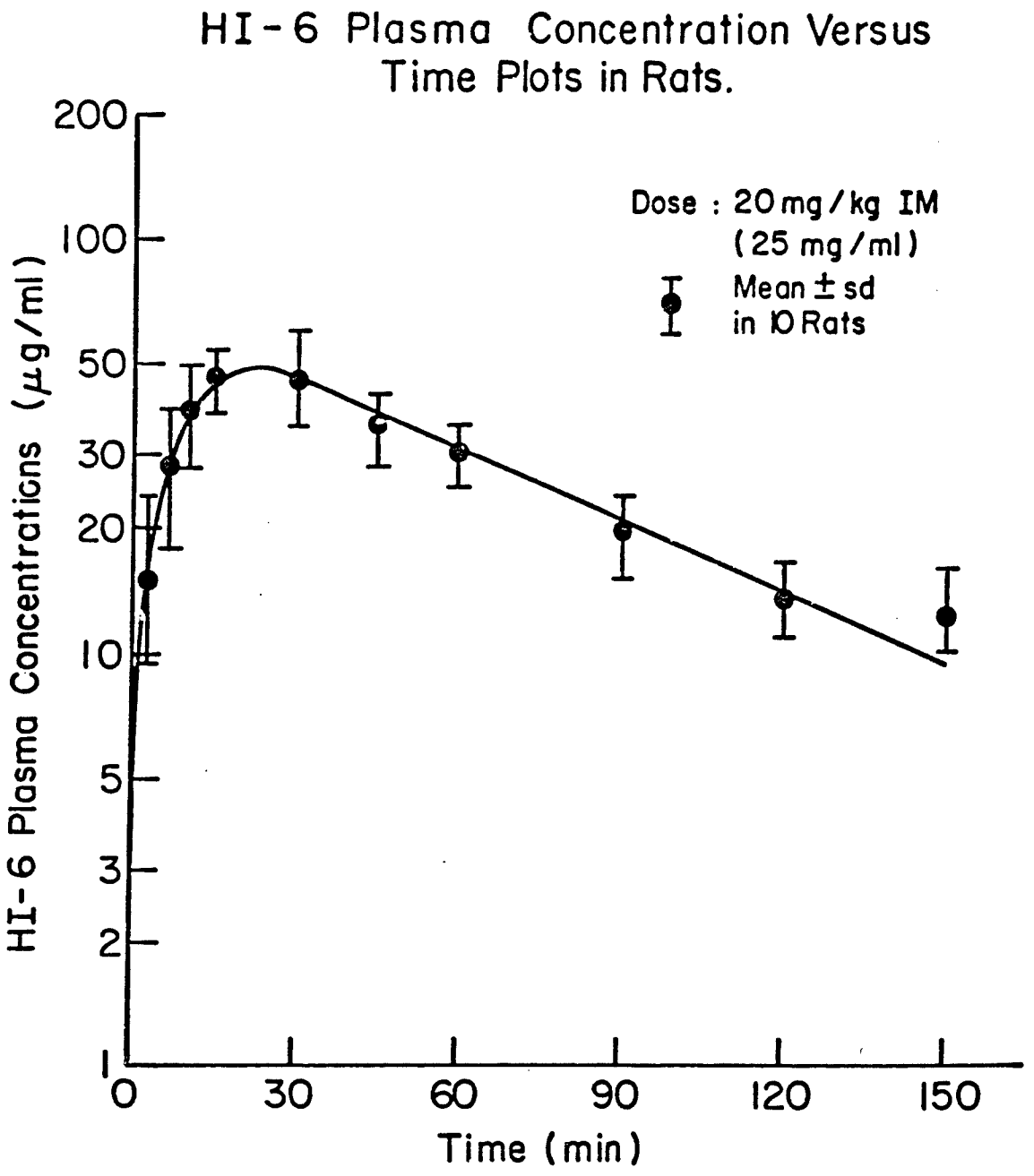
HI-6 Plasma Concentration Versus Time Plots in Rats.
Dose : 20 mg/kg IM
(25 mg/ml)
Mean ± sd
in 10 Rats
HI-6 Plasma Concentrations (μg/ml)
200
100
50
30
20
10
5
3
2
1
0
30
60
90
120
150
Time (min)

TABLE I

DOSING SCHEDULE FOR
HI-6 PHARMACOKINETIC STUDY IN DOGS

DOG	STUDY I	STUDY II	STUDY III
1	A	B	C
2	B	C	A
3	C	A	B
4	C	B	A
5	B	A	C
6	A	C	B
7	A	B	C

DOSE TYPE	DOSE	DOSE SOLUTION STRENGTH
A	20 mg/kg IV	250 mg/ml
B	20 mg/kg IM (small volume)	250 mg/ml
C	20 mg/kg IM (large volume)	25 mg/ml

TABLE II

DOSING SCHEDULE FOR HI-6 PHARMACOKINETIC STUDY IN RATS

Week of	Monday	Wednesday	Friday
Nov. 16tn			F_1, F_2
Nov. 23rd	E_1, E_2	F_1, F_2	D_1, D_2
Nov. 30th	F_1, F_2	D_1, D_2	E_1, E_2
Dec. 7th	E_1, E_2	D_1, D_2	F_1, F_2
Dec. 14th	D_1, D_2	F_1, F_2	E_1, E_2
Dec. 21st	D_1, D_2	E_1, E_2	

Dose Type	Dose	Dose Strength
D	20 /mg/k IV	125 mg/ml HI-6
E	20 mg/kg IM	125 mg/ml HI-6
F	20 mg/kg IM	25 mg/ml HI-6

Sampling: Series subscript 1: 3, 7, 10, 15, 30, 90 min

Series subscript 2: 30, 45, 60, 90, 120, 150 min.

TABLE III

PLASMA CONCENTRATIONS OF HI-6 (μg/ml) IN DOGS GIVEN 20 mg/kg DOSE (250 μg/ml) INTRAVENOUSLY

Type A

Dog	Time (min) 2	7 (5)	10	15	20	30	45	60	75	90	105	120
1	--[a]	69.74	63.81	53.73	44.07	33.90	22.46	15.60	--	8.49	--	4.16
2	77.51	60.11	59.59	51.66	46.29	37.33	26.58	20.70	15.83	11.84	9.18	7.39
3	93.60	---	71.76	56.19	53.68	43.14	33.60	26.82	21.80	15.77	14.26	10.75
4	82.98	68.46	61.33	54.97	---	41.47	27.72	31.97	19.83	15.50	12.95	10.66
5	97.24	70.91	66.57	57.11	52.00	41.01	31.30	24.66	20.06	15.20	12.39	9.32
6	104.89	74.14	62.35	56.71	46.71	38.25	25.44	21.85	15.95	12.11	10.06	8.26
7	102.23	71.32	61.36	53.19	49.62	35.32	---	28.42	21.27	16.42	12.84	10.65
Mean	93.08	69.11	63.82	54.79	48.73	38.63	27.85	24.29	19.12	13.62	11.95	8.74
±S.D.	10.82	4.80	4.14	2.02	3.68	3.40	4.04	5.43	2.61	2.89	1.93	2.41

[a]Sample not collected or volume too small for analysis

TABLE IV

PLASMA CONCENTRATIONS OF Hi-6 (μg/ml) IN DOGS GIVEN 20 mg/kg (250 mg/ml) INTRAMUSCULARLY

Type B

Dog	Time (min) 5	10	15	20	30	45	60	75	90	105	120	(180)
1	47.22	54.14	51.84	48.76	39.53	29.28	21.59	16.21	12.88	10.06	-- [a]	
2	31.11	34.92	47.12	51.70	43.56	37.21	27.80	15.60	9.25	--	9.25	3.40
3	35.80	35.54	38.61	39.12	41.17	36.56	31.70	24.02	19.67	17.11	14.04	
4	21.59	33.09	36.67	39.22	39.22	35.65	28.75	22.87	19.03	15.71	12.13	
5	21.95	25.66	33.36	36.18	40.55	35.16	26.43	21.55	17.19	14.11	9.99	
6	31.65	36.35	39.81	41.55	40.06	36.16	28.18	21.99	19.28	16.31	13.83	
7	37.14	45.80	48.09	45.29	43.25	35.36	25.68	20.34	16.01	12.70	10.15	
Mean	32.35	37.93	42.21	43.12	41.05	35.05	27.16	20.37	16.19	14.33	11.57	
±S.D.	8.96	9.27	6.82	5.66	1.73	2.64	3.12	3.26	3.87	2.63	2.07	

[a] Sample not collected or volume too small for analysis

TABLE V

PLASMA CONCENTRATIONS OF HI-6 (μg/ml) IN DOGS GIVEN 20 mg/kg DOSE (25 mg/ml) INTRAMUSCULARLY

Type C

Dog	Time (min) 5	10	15	20	30	45	60	75	90	105	120
1	29.60	38.34	40.40	39.11	38.60	32.94	27.80	22.91	18.03	15.71	13.65
2	39.62	55.69	60.71	54.19	46.65	39.12	27.82	21.04	18.03	15.27	11.25
3	47.47	49.79	54.46	52.61	44.65	37.72	29.25	23.90	19.50	15.90	12.82
4	29.02	42.15	45.75	45.50	41.63	33.40	26.96	21.30	17.43	15.12	13.57
5	40.73	45.86	47.39	50.47	43.55	39.45	30.99	21.76	18.69	15.10	12.54
6	-- [a]	41.42	42.16	45.13	41.42	37.46	28.30	23.85	19.14	15.92	13.45
7	16.64	42.04	38.33	41.30	39.32	34.87	28.68	21.75	17.79	---	12.35
Mean	33.85	45.04	47.03	46.90	42.26	36.42	28.54	22.36	18.37	15.50	12.80
±S.D.	10.99	5.94	8.04	5.71	2.88	2.67	1.30	1.19	0.76	0.38	0.86

[a] Sample not collected or volume too small for analysis

TABLE VI

PHARMACOKINETIC PARAMETERS OF H1-6 in DOGS
GIVEN 20 mg/kg DOSE (250 mg/ml) INTRAVENOUSLY

Type A

Dog	Weight kg	Dose mg	$\alpha(min^{-1})$	$t1/2\alpha$(min)	$\beta(min^{-1})$	$t1/2\beta$(min)	AUC* µg/ml/min	Cl ml/min/kg	Vd l/kg	% Excreted Unchanged	Cl_R ml/min/kg
1	9.0	180	0.033	21.00	0.008	86.63	3041.33	6.56	0.82	37.61	2.49
2	8.8	176	0.638	1.09	0.020	34.65	3431.55	5.80	0.29	71.16	4.13
3	9.2	184	0.102	6.79	0.015	46.20	4544.69	4.35	0.29	66.25	2.88
4	9.9	198	0.148	4.68	0.016	43.31	4264.11	4.65	0.29	50.39	2.34
5	8.8	176	0.215	3.22	0.017	40.77	4153.49	4.77	0.28	82.96	3.96
6	8.8	176	0.189	3.67	0.019	36.47	3698.03	5.46	0.29	59.55	3.25
7	8.8	176	0.187	3.71	0.014	49.50	4368.74	4.55	0.33	60.47	2.75
Mean	9.04	180.86	0.216	6.309	0.016	48.219	3928.85	5.16	0.37	61.20	3.11
±S.D.	0.41	8.15	0.196	6.698	0.004	17.715	551.35	0.81	0.20	14.58	0.70

* Area under the plasma concentration versus time curve.

TABLE VII

PHARMACOKINETIC PARAMETERS OF H1-6 in DOGS
GIVEN 20 mg/kg DOSE (250 mg/ml) INTRAMUSCULARLY

Type B

Dog	Weight kg	Dose mg	Ka(min^{-1})	t1/2abs(min)	K(min^{-1})	t1/2(min)	*AUC µg/ml/min	Cl ml/min/kg	VD l/kg	% Excreted Unchanged	Cl_R ml/min/kg
1	9.6	192	0.271	2.56	0.019	36.47	3312.10	6.04	0.32	79.23	4.79
2	8.5	170	0.080	8.66	0.025	27.72	3491.75	5.77	0.23	77.85	4.49
3	9.4	188	0.056	12.38	0.015	46.20	4245.48	4.68	0.31	52.61	2.46
4	8.8	176	0.082	8.45	0.014	49.50	3974.73	5.00	0.36	77.64	3.88
5	9.3	186	0.077	9.00	0.017	40.76	3507.38	5.70	0.34	11.74	0.67
6	9.2	184	0.077	9.00	0.014	49.50	4192.69	4.78	0.34	12.36	0.59
7	9.4	188	0.118	5.87	0.017	40.76	3767.14	5.32	0.31	71.48	3.80
Mean	9.17	183.43	0.109	7.989	0.017	41.559	3784.47	5.33	0.32	54.70	2.95
±S.D.	0.39	7.72	0.074	3.054	0.004	7.814	365.44	0.53	0.04	30.52	1.75

* Area under the plasma concentration versus time curve

TABLE VIII

PHARMACOKINETIC PARAMETERS OF H1-6 IN
DOGS GIVEN 20 mg/kg DOSE (25 mg/ml) INTRAMUSCULARLY

Type C

Dog	Weight kg	Dose mg	Ka(min^{-1})	t1/2abs(min)	K(min^{-1})	t1/2(min)	*AUC µg/ml/min	Cl ml/min/kg	Vd l/kg	% Excreted Unchanged	Cl_R ml/min/kg
1	9.8	198	0.122	5.68	0.013	53.31	3986.90	5.10	0.39	80.88	4.12
2	8.5	170	0.247	2.81	0.016	43.31	4039.03	4.94	0.31	45.82	2.26
3	9.5	190	0.133	5.21	0.015	46.20	4437.50	4.51	0.30	80.42	3.63
4	9.7	194	0.177	3.92	0.014	49.50	4157.89	4.85	0.35	76.73	3.72
5	9.7	194	0.080	8.66	0.016	43.31	4258.63	4.72	0.30	65.03	3.07
6	9.4	188	0.082	8.45	0.014	49.50	4102.21	4.89	0.35	63.62	3.11
7	9.2	184	0.108	6.42	0.014	49.50	3991.87	5.00	0.36	76.61	3.83
Mean	9.40	188.29	0.136	5.879	0.015	47.804	4139.15	4.86	0.34	69.87	3.39
±S.D.	0.45	9.27	0.059	2.175	0.001	3.695	163.25	0.19	0.03	12.69	0.63

* Area under the plasma concentration versus time curve.

TABLE IX

PLASMA CONCENTRATIONS OF HI-6 (μg/ml) IN RATS GIVEN 20 mg/kg DOSE (125 mg/ml) INTRAVENOUSLY

	Type D				Time (min)					
Rat	3	7	10	15	30	45	60	90	120	150
9	97.46	71.43	51.81	36.73	25.78	--	--	10.70	--	--
10	--	--	--	--	32.80	25.99	22.58	18.65	15.04	11.5
13	92.80	65.41	52.55	36.68	22.93	--	--	--	--	--
14	--	--	--	--	28.07	20.01	17.34	--	--	--
19	161.36	106.17	91.04	78.38	58.70	--	--	27.06	--	--
20	--	--	--	--	92.53	95.59	95.20	87.09	74.13	68.7
23	165.86	94.69	80.49	59.17	36.78	--	--	16.14	--	--
24	--	--	--	--	77.46	59.26	47.39	34.15	26.65	23.4
29	184.96	98.16	89.64	69.03	43.29	--	--	12.91	--	--
30	--	--	--	--	30.32	27.23	21.52	13.78	12.23	9.6
Mean	140.49	87.17	73.11	56.00	39.57	33.12	27.21	19.06	17.97	14.86
±S.D.	42.38	17.75	19.53	18.88	17.87	17.71	13.64	8.52	7.64	7.49

TABLE X

PLASMA CONCENTRATIONS OF HI-6 (μg/ml) IN RATS

GIVEN 20 mg/kg DOSE (125 mg/ml) INTRAMUSCULARLY

Type E	Time (min)									
Rat	3	7	10	15	30	45	60	90	120	150
5	24.68	40.19	49.06	47.85	42.06	--	--	19.72	--	--
6	--	--	--	--	48.22	39.16	28.69	21.87	12.80	10.00
15	15.32	48.86	54.01	47.03	29.63	--	--	--	--	--
16	--	--	--	--	38.58	22.93	16.78	--	--	--
17	19.84	36.95	40.51	44.76	34.18	--	--	11.23	--	--
18	--	--	--	--	50.59	32.99	21.81	13.61	8.96	6.09
27	19.12	39.02	44.53	60.18	57.76	--	--	32.06	--	--
28	--	--	--	--	50.03	45.30	44.53	40.37	30.33	26.46
31	12.04	25.68	33.23	43.58	52.68	--	--	34.20	--	--
32	--	--	--	--	35.16	28.97	22.87	13.20	12.33	6.23
33	14.85	30.27	24.01	33.17	33.12	--	--	19.61	16.08	12.21
Mean	17.64	36.83	40.89	46.10	42.91	32.65	26.94	22.87	16.10	12.20
±S.D.	4.50	8.10	10.92	8.69	9.39	9.91	10.71	10.34	8.35	8.38

TABLE XI

PLASMA CONCENTRATIONS OF HI-6 (μg/ml) IN RATS

GIVEN 20 $mg/_{kg}$ DOSE (25 mg/ml) INTRAMUSCULARLY

Type F	Time (min)									
Rat	3	7	10	15	30	45	60	90	120	150
3	16.92	29.81	35.70	47.57	36.82	--	--	10.19	--	--
4	--	--	--	--	--	40.37	32.43	23.65	16.73	15.89
7	30.62	44.61	45.63	51.43	46.09	--	--	16.72	--	--
8	--	--	--	--	--	24.54	22.43	17.55	11.93	7.42
11	5.74	17.93	26.92	32.93	45.92	--	--	19.48	--	--
12	--	--	--	--	48.09	41.48	34.46	18.65	11.11	--
21	11.96	23.05	49.14	50.60	61.41	--	--	21.59	--	--
22	--	--	--	--	26.46	31.23	26.46	15.26	12.05	12.83
25	10.91	28.49	34.19	51.48	47.81	--	--	22.89	--	--
26	--	--	--	--	48.68	39.31	35.45	25.11	15.64	13.42
Mean	15.23	28.78	38.32	46.80	45.16	35.39	30.25	19.11	13.49	12.39
±S.D.	9.48	10.03	9.01	7.92	10.09	7.28	5.59	4.47	2.51	3.57

TABLE XII

PHARMACOKINETIC PARAMETERS OF H1-6 in RATS
GIVEN 20 mg/kg DOSE (125 mg/ml) INTRAVENOUSLY

TYPE D

RAT	WEIGHT (kg)	DOSE (mg)	$\alpha(min^{-1})$	$t1/2\alpha$(min)	$\beta(min^{-1})$	$t1/2\beta$(min)	* AUC µg/ml/min	Cl ml/min/kg	Vd l/kg	% Excreted Unchanged	Cl_R ml/min/kg
9+10	0.540	10.8	0.166	4.72	0.007	99.00	5238.58	3.82	0.556	65.98	2.52
13+14	0.480	9.6	0.140	4.95	0.009	77.00	3731.66	5.36	0.602	57.69	3.09
19	0.520	10.4	0.280	2.48	0.014	49.50	6788.19	2.95	0.206	83.75	2.47
23	0.520	10.4	0.233	2.97	0.017	40.77	4398.66	4.56	0.272	104.83	4.78
24	0.520	10.4	--	--	0.012	57.75	6665.06	3.00	0.256	78.56	2.36
29+30	0.610	12.2	0.132	5.25	0.010	69.30	4983.49	4.01	0.393	76.22	3.06
Mean	0.532	10.63	0.190	4.074	0.012	65.55	5299.44	3.95	0.381	77.84	3.05
±S.D.	0.043	0.86	0.064	1.258	0.004	20.96	1222.33	0.926	0.166	16.21	0.90

* Area under the plasma concentration versus time curve

TABLE XIII

PHARMACOKINETIC PARAMETERS OF H1-6 in RATS
GIVEN 20 mg/kg DOSE (125 mg/ml) INTRAMUSULARLY

TYPE E

Rat	Weight kg	Dose mg	Ka(min^{-1})	t1/2abs(min)	K(min^{-1})	t1/2(min)	* AUC µg/ml/min	Cl ml/min/kg	Vd l/kg	% Excreted Unchanged	Cl_R ml/min/kg
5+6	0.470	9.0	0.169	4.10	0.013	53.31	4734.12	4.05	0.311	84.57	3.43
15+16	0.580	11.6	0.413	1.68	0.024	28.88	2568.61	7.79	0.323	76.23	5.94
17+18	0.515	10.3	0.126	5.46	0.019	36.47	3498.05	5.73	0.307	77.63	4.45
27+28	0.535	10.7	0.176	3.9	0.007	99.00	9618.06	2.08	0.294	70.11	1.46
31	0.490	9.8	0.067	10.34	0.011	63.00	6793.27	2.94	0.260	77.24	2.27
32	0.440	8.8	--	--	0.014	49.50	2517.18	7.95	0.568	61.36	4.88
33	0.580	11.6	0.299	2.32	0.008	86.63	4842.18	4.13	0.517	87.33	3.61
Mean	0.516	10.26	0.208	4.640	0.014	59.54	4937.49	4.95	0.369	76.35	3.72
±S.D.	0.053	1.134	0.126	3.10	0.006	25.54	2548.52	2.29	0.121	8.70	1.53

* Area under the plasma concentration versus time curve

TABLE IV

PHARMACOKINETIC PARAMETERS OF H1-6 in RATS
GIVEN (20 mg/kg) DOSE (25 mg/ml) INTRAMUSCULARLY

PE F

E	Weight kg	Dose mg	Ka(min^{-1})	t1/2abs(min)	K(min^{-1})	t1/2(min)	* AUC µg/ml/min	Cl ml/min/kg	Vd l/kg	% Excreted Unchanged	Cl_R ml/min/kg
)	0.510	10.2	0.126	5.50	0.010	69.30	5768.45	3.47	0.353	57.83	2.01
3	0.465	9.3	0.126	5.50	0.016	43.31	3841.92	5.21	0.323	62.29	3.25
-12	0.480	9.6	0.040	17.33	0.027	25.67	3764.15	5.31	0.201	85.58	4.54
	0.450	9.0	0.060	11.55	0.024	28.88	4657.31	4.29	0.176	90.05	3.86
	0.430	8.6	--	--	0.014	49.50	3190.43	6.27	0.449	92.29	5.79
-26	0.495	9.9	0.118	5.87	0.012	57.75	5392.95	3.71	0.303	87.7	3.25
n	0.472	9.43	0.094	9.15	0.017	45.74	4435.87	4.71	0.301	79.29	3.78
D.	0.029	0.589	0.041	5.25	0.007	16.77	1009.55	1.073	0.101	15.13	1.29

ea under the plasma concentration versus time curve

APPENDIX I

APPENDIX 1

MICROBIOLOGY REQUISITION AND REPORT
ST. BONIFACE GENERAL HOSPITAL

6	6	0

98810

PATIENT No. PHARMACY
NAME
AGE H1-6
SEX
DOCTOR
ROOM NO
DATE 81-11-23

10 IN P ☐ · 20 OUT P ☐ · 30 REF IN ☐ · 40 PERSONNEL ☐ · 41 ENVI ☒ · 42 QUA C ☐ · 50 RES ☐ · 99 OTHER ☐ · STAT ☐

CLINICAL IMPRESSION
Samples for Rel Study (preventive individ.)

ANTIBIOTICS (DATE STARTED & TYPE)

SIGNATURE

BLOOD COLLECTED BY: 952 ☐ MICROB. 953 ☐ HEMAT. 954 ☐ HEMAT. 998 ☐ CHEM. 999 ☐ CHEM.

SPECIMEN TYPE:
(Specify origin in detail)

Swab of____
Secretions of____
Aspirated Fluid of____
Feces____
Urine: M.S.U. ☐ CATH. ☐ CYSTO. ☐
Tissue of____
Other:____

PLEASE CHECK TEST(S) REQUIRED

CEREBROSPINAL FLUID

- ☐ Cell count
- ☐ Non-TB, smear & culture
- ☐ TB, smear & culture
- ☐ India Ink wet mount
- ☐ Fungus, culture
- ☐ V.D.R.L. re syphilis
- ☐ Protein____mgm% (N=15-45)
- ☐ Sugar____mgm% (N=50-70)
- ☐ Xanthochromia

MISCELLANEOUS TESTS

- ☐ Antibiotic Sensitivity
- ☐ Non-TB, smear & culture
- ☐ TB, smear & culture
- ☐ Candida, smear & culture
- ☐ Fungus, microscopic & culture
- ☐ G.C., smear & culture
- ☐ Diphtheria, smear & culture
- ☐ Pertussis, smear & culture
- ☐ Gram stain____only
- ☐ Acid fast stain____only
- ☐ Wet mount re microscopic elements
- ☐ White cell count on fluids (in citrate)
- ☐ Parasites
- ☐ Scotch tape re Pinworms
- ☐ Trichomonas
- ☒ Environmental Sterility Test
- ☐ Pregnancy Slide Test

VIRAL STUDIES
(Prov. Lab. History form must be completed and accompany specimen)
- ☐ Blood
- ☐ Other____ (specify)

BLOOD, SERUM

see BIOCHEM ☐ see HEMAT.
- ☐ Blood culture
- ☐ V.D.R.L. re syphilis
- ☐ Typhoid & Paratyphoid slide agglutination
- ☐ Brucella Slide agglutination
- ☐ Heterophile Antibodies
- ☐ C-Reactive Protein
- ☐ Anti-Streptolysin 'O' Titre
- ☐ Rheumatoid Arthritis (slide)
- ☐ Thyroglobulin Antibody Test
- ☐ Thyroid Microsomal Antibody
- ☐ Cold Agglutinins
- ☐ Serum Bactericidal Level
- ☐ Serum Gentamicin Level

TEST(S) NOT LISTED
(write in)

[signature]

REPORT: L = LIGHT M = MODERATE H = HEAVY

Gram Smear: Pus____ G(+) Cocci____ G(-) Cocci____
G(+- Bacilli____ G(-) Bacilli____
No Bacteria____ Yeast:____ With Hyphae____ No Hyphae

- ☒ NO GROWTH ISOLATED x2
- ☐ NORMAL FLORA
- ☐ NO G.C. ISOLATED
- ☐ NO ENTERIC PATHOGENS

URINE COLONY COUNT: ☐ LESS THAN 1,000 bact./cc ☐ LESS THAN 10,000 bact./cc

S = SENSITIVE R = RESISTANT

PENICILLIN	AMOXACILLIN-AMPICILLIN	TETRACYCLINE GROUP	CHLORAMPHENICOL	CEPHALOSPORINS	ERYTHROMYCIN	NITROFURAN GROUP	SULFA	COTRIMOXAZOLE	NALIDIXIC ACID	KANAMYCIN	GENTAMICIN		CLINDAMYCIN	OXA___, CLOXA DICLOXACILLIN	CARBENICILLIN
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Signature BL Date Nov 30/81

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MICROBIOLOG

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